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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re New U.S. Continuation Appln. of:	
Pascal DESMAZEAU et al.	Parent Group Art Unit: 1653
Application No.: 10/790,260	Parent Examiner: D. Lukton
Filed: March 2, 2004	
For: STREPTOGRAMIN DERIVATIVES, PREPARATION METHOD AND COMPOSITIONS CONTAINING SAME	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION UNDER 37 C.F.R. 1.132

I, Dr. Nadine BERTHAUD, declare and state that:

1. I am a French citizen, residing at 20 rue de Marne, 94140 Alfortville, France.

2. I have been awarded the degree of Doctor of Veterinary Medicine from Ecole Nationale Vétérinaire de Maisons-Alfort, and have a diploma from the Institut Pasteur for a course entitled Systematic Microbiology.

3. I have been employed by Aventis Pharma S.A., formerly Rhone-Poulenc Rorer, S.A., ("Aventis") since 1977 and until 2003 I was the Head of Antibacterial Microbiology in the Infectious Disease Group at Aventis. During this employment at

Aventis, I have been engaged in applied research and development regarding potential antibacterial compounds and I was responsible for the evaluation of the *in vitro* and *in vivo* activity of new antibacterial agents.

4. Given my education and experience, particularly in the area of antibacterial compounds, I consider myself qualified to provide the following testimony based on the below-described experiments related to U.S. Patent Application No. 10/790,260 ("the '260 application"), conducted by me or under my direct supervision.

5. Given my education and experience, I also consider myself qualifed to provide the following testimony concerning the common names of Streptogramin A compounds.

6. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the '260 application or any patent issuing thereon.

I. Testing

Streptogramin compounds according to general formula (I) in U.S. Patent Application No. 10/790,260, were tested for *in vitro* and *in vivo* activity against the bacteria *S.aureus (in vitro), S.aureus Schiclia (in vitro)* and *S.aureus IP8203 (in vivo)*. As described further below, the testing included measurement of the activity of compounds according to general formula (I) tested (1) *in vitro* against exemplary

bacteria (*S.aureus, S.aureus Schiclia*) to determine a minimum effective concentration, both individually and in combination with pristinamycin IIB ("PIIB"), and (2) *in vivo* against an exemplary bacteria (*S.aureus IP8203*) to determine a 50% curative dose in combination with each of dalfopristin and PIIB, via subcutaneous and oral routes, respectively.

The testing procedures were as follows:

***In vitro* bacteriostatic activity**

The bacteriostatic activity of the compounds of general formula (I) of the '260 application was determined according to the U.S. standards (Antimicrobial Susceptibility Testing: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. 1992 National Committee for Clinical Laboratory Standards, M7-A2, Villanova, PA.).

Two-fold dilutions of the 1280 mg/l antibacterial stock solution tested were added to molten Mueller-Hinton agar supplemented with 25 mg/l Mg++ and 50 mg/l CA++ (1 part of antibacterial solution for 9 parts of liquid agar), then poured into plates. A multipoint inoculator was used to apply spots of about 10^4 colony forming units (cfu) of each strain tested onto agar. After inoculation, plates were incubated 18 hours at 37°C.

The minimum inhibitory concentration ("MIC") was defined as the lowest concentration (µg/ml) that completely inhibited the growth of bacteria.

For the combination treatments (*i.e.*, test compound in combination with PIIB), a 30/70 weight/weight ratio of test compound to PIIB was used.

***In vivo* antibacterial activity in the model of *staphylococcus aureus* mouse septicemia**

Mice (6 to 8 per group) were inoculated intraperitoneally with 0.5 ml of the bacterial strain cultured under shaking in Brain Heart Infusion at 37°C and diluted in 7.5% porcine mucin so as to obtain about 10^6 cfu/ml. Under these conditions, infected untreated controls die in 24 to 48 hours.

Each compound tested was administered by the subcutaneous (s.c.) or the oral (p.o.) routes twice on the day of inoculation, the first dose being given 1 hour after infection and the second dose 6 hours after infection.

The test compositions contained a compound according to general formula (I) with PIIB or dalfopristin, as indicated in the following table, in a 30/70 weight/weight ratio of the formula (I) compound to PIIB or dalfopristin.

The vehicle was an aqueous solution or a suspension in 0.9% NaCl aqueous solution added with 0.1% polysorbate 80 (Prolabo). The administered volume was 1 ml/mouse per treatment.

Three to 6 doses of up to 150 mg/kg were used.

The Curative Dose 50 ("DC_{50}") (mg/kg), calculated 7 days post infection, was defined as the dose which protected 50% of the infected treated mice from death when all the infected untreated controls died.

II. Results

The results of the testing procedures above as applied to compounds according general formula (I) of the '260 application are presented in the following table. For reference, results for PIIB and dalfopristin alone are also provided. In the table, the

example numbers refer to the compounds of the corresponding examples in the '260 application.

Example No.	*In Vitro S.aureus 209P* MIC (µg/ml)		*In Vitro S.aureus Schiclia* MIC (µg/ml)		*In Vivo S.aureus IP8203* DC_{50} (mg/kg)	
	Compound Alone	With PIIB	Compound Alone	With PIIB	S.C. with dalfopristin	P.O. with PIIB
1	8	0.5	>128	2	120	32
2	2	0.25	>128	1	28	32
3	2	0.25	>128	1	32	28
4	2	0.25	>128	1	32	32
5	4	0.25	128	1	30	30
6	16	1	>128	2	38	95
7	64	1	>128	4	75	36
8	8	1	>128	2	32	90
9	16	1	>128	2	5	100
10	64	4	>128	4	46	150
11	4	0.25	>128	0.5	90	100
12	2	0.5	>128	0.5	36	100
13	128	1	>128	2	85	100
14	2	0.25	>128	4	<5	42
15	4	0.5	>128	1	42	50
16	4	0.5	>128	1	42	32
17	4	0.25	>128	1	46	110
18	8	0.5	>128	1	36	34
19	8	0.5	>128	1	36	34
20	4	0.5	128	1	40	50
21	8	0.5	>128	1	90	75
22	128	2	>128	4	100	110
23	>128	1	>128	2	>150	100
24	8	1	>128	2	36	44
25	4	0.5	>128	1	32	26

Example No.	*In Vitro S.aureus 209P* MIC (µg/ml)		*In Vitro S.aureus Schiclia* MIC (µg/ml)		*In Vivo S.aureus IP8203* DC_{50} (mg/kg)	
	Compound Alone	With PIIB	Compound Alone	With PIIB	S.C. with dalfopristin	P.O. with PIIB
26	2	0.25	>128	1	40	32
27	32	1	>128	4	32	110
28	4	0.25	>128	1	32	40
29	4	0.5	>128	1	10	110
30	4	0.25	>128	1	32	15
31	16	1	>128	2	15	100
32	4	0.25	>128	0.5	120	44
33	4	0.25	>128	0.5	8	50
34	64	2	>128	2	85	110
35	8	1	>128	2	8	50
PIIB alone		4		4		> 300
dalfopristin alone					> 300	

III. **Analysis**

As shown by the results, *in vitro*, streptogramin compounds according to general formula (I) have proven active against *Staphylococcus aureus* 209P at concentrations of as low as 1 µg/ml, and in combination with pristinamycin IIB, have proven active at concentrations of 0.25 to 10 µg/ml. Additionally, *in vitro*, compounds according to general formula (I) in combination with PIIB have proven effective against *S. aureus Schiclia* at concentrations ranging from 0.5 to 4 µg/ml. In the combination treatments, the results show that, in nearly every instance, the activity of the combination is enhanced over either the streptogramin formula (I) compound or PIIB, when tested individually. For instance, against *S.aureus 209P*, the MIC activities of compound 1 and

PIIB, tested individually, were 8 and 4 µg/ml, respectively, while in combination the MIC activity was 0.5 µg/ml. Further, against *S.aureus Schiclia*, the MIC activities of compound 1 and PIIB, tested individually, were >128 and 4 µg/ml, respectively, while in combination the MIC activity was 2 µg/ml.

In vivo, streptogramin compounds according to general formula (I) have proven effective against *Staphylococcus aureus* IP 8203 test infections in mice in subcutaneous doses of 25 to 150mg/kg combined with dalfopristin, and with orally administered doses of 15 to 150mg/kg combined with pristinamycin IIB.* These results can be compared to an activity of > 300 mg/kg for dalfopristin and pristinamycin IIB, when each is tested individually in analogous *in vivo* tests. Thus, the combination treatments were more potent in their DC_{50} than both dalfopristin and pristinamycin IIB taken individually.

* For compound 23, the *in vivo* activity (DC_{50}) with dalfopristin was only determined to be greater than 150 mg/kg. Higher doses were not tested to more precisely determine the DC_{50}.

IV. Common names of Streptogramin A compounds

In my experience, streptogramin A compounds are often identified by common names, and in my opinion one skilled in the art would understand the reference to a streptogramin A compound based on its common name. In this regard, I am aware of several references that expressly refer to streptogramin A compounds by their common names. *See, e.g.*, J.C. Barrière et al., Current Pharmaceutical Design, 4, 155-180 (1998) (referring at pg. 156 to, *inter alia*, pristinamycin II_A, II_B, II_C, II_D, II_E, II_F, and II_G); V. Blanc et al., J. Bacteriology 177 (18), 5206-5214 (1995) (discussing streptogramin compounds including pristinamycin II_A and II_B).

Date: July 23rd 2004 By: _____
Nadine BERTHAUD